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New distribution record of the brine shrimp *Artemia* (Crustacea, Branchiopoda, Anostraca) in Tunisia

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The genus *Artemia* Linnaeus, 1758 comprises a number of sexual species and several parthenogenetic populations. In the New World, only two sexual species are present: *Artemia franciscana* (Kellogg, 1906) and *Artemia persimilis* (Piccinelli and Prosdocimi, 1968). In the Old World, where both sexual and parthenogenetic populations occur, five sexual species have been described: *Artemia salina* (Leach, 1819), *Artemia urmiana* (Günther, 1890), *Artemia sinica* (Cai, 1989), *Artemia* sp. (Pilla and Beardmore, 1994) and *Artemia tibetiana* (Abatzopoulos et al., 1998).

Artemia is an anostracan crustacean, occurring in every continent except Antarctica (Triantaphyllidis et al. 1998). It is a typical inhabitant of inland salt lakes, coastal salt lagoons and solar saltworks (Persoone and Sorgeloos 1980). The brine shrimp is able to overcome the severe physiological demands imposed by these habitats, due to a set of various adaptations, the most salient of which is probably an interchangeable (diapausing cysts versus nauplii) life cycle. In fact, the life cycle of *Artemia* can begin as an embryo within a dormant cyst. Depending on environmental conditions, embryos can enter into diapause and arrested development for many years and are capable of surviving a very wide range of environmental conditions (Clegg and Trotman 2002). *Artemia* was first described from saltpans of Lymington, Hampshire (England) by Schlösser in 1755 (Kuenen and Baas-Becking 1938).

Leach (1819) named this taxon *Artemia salina* (Artom 1931), but Bowen and Sterling (1978) suggested the binomen *Artemia salina* to be restricted to the extinct population at Lymington (England) and use the binomen *Artemia tunisiana* to describe bisexual populations in the Mediterranean area. Browne (1988) confirmed that all strains sampled from the Mediterranean region are able to interbreed and should be classified as *Artemia salina*. Mura (1990) based on the comparison of the frontal knob morphology, using scanning electron microscopy, reported that in one hand there are no significant differences between Lymington and North African *Artemia* populations and on the other hand, North African populations cannot be separated from the Italian populations.

Lately, Barigozzi and Baratelli (1993) suggested to keep the binomen *Artemia salina* for the Italian populations, while the North African populations could be named *Artemia tunisiana*. The confusion continued until Triantaphyllidis et al. (1997) using Amplified Fragment Length Polymorphism (AFLP), confirmed that all strains from the Mediterranean Basin should be classified as *Artemia salina*, and that *Artemia* from Mediterranean Basin can be grouped in two subclusters (the Eastern Mediterranean basin group and the Western Mediterranean basin group).

However, the Western Mediterranean Basin shows the unfortunate event of the presence of the American species *Artemia franciscana*, causing a great change in the *Artemia* populations' biodiversity in this region (Amat et al. 2005, 2007). This event was initially stated in Portugal (Hontoria et al. 1987 in Amat et al. 2007), in France (Thiery and Robert 1992), in Spain, in Morocco and in Italia (Amat et al. 2007), but there

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are no information about the existence of the exotic brine shrimp *A. franciscana* in Tunisia.

The presence of *Artemia* in Tunisia was first reported by Seurat (1921) and Gauthier (1928) in Chott Ariana and Sabkhet Sidi El Hani, respectively. Later, Ben Abdelkader (1985), Sorgeloos et al. (1986), Romdhane (1994), Triantaphyllidis et al. (1998) and Romdhane et al. (2001) announced the occurrence of *Artemia* populations in 10 other sites. Since then, *Artemia* reported from the Tunisian sites was used as a biological material and as a reference for several scientists (e.g. Léger et al. 1986; Abatzopoulos et al. 2002; Bossier et al. 2004). From then no other *Artemia* sites have been announced in the review of the distribution of the genus *Artemia* in Tunisia.

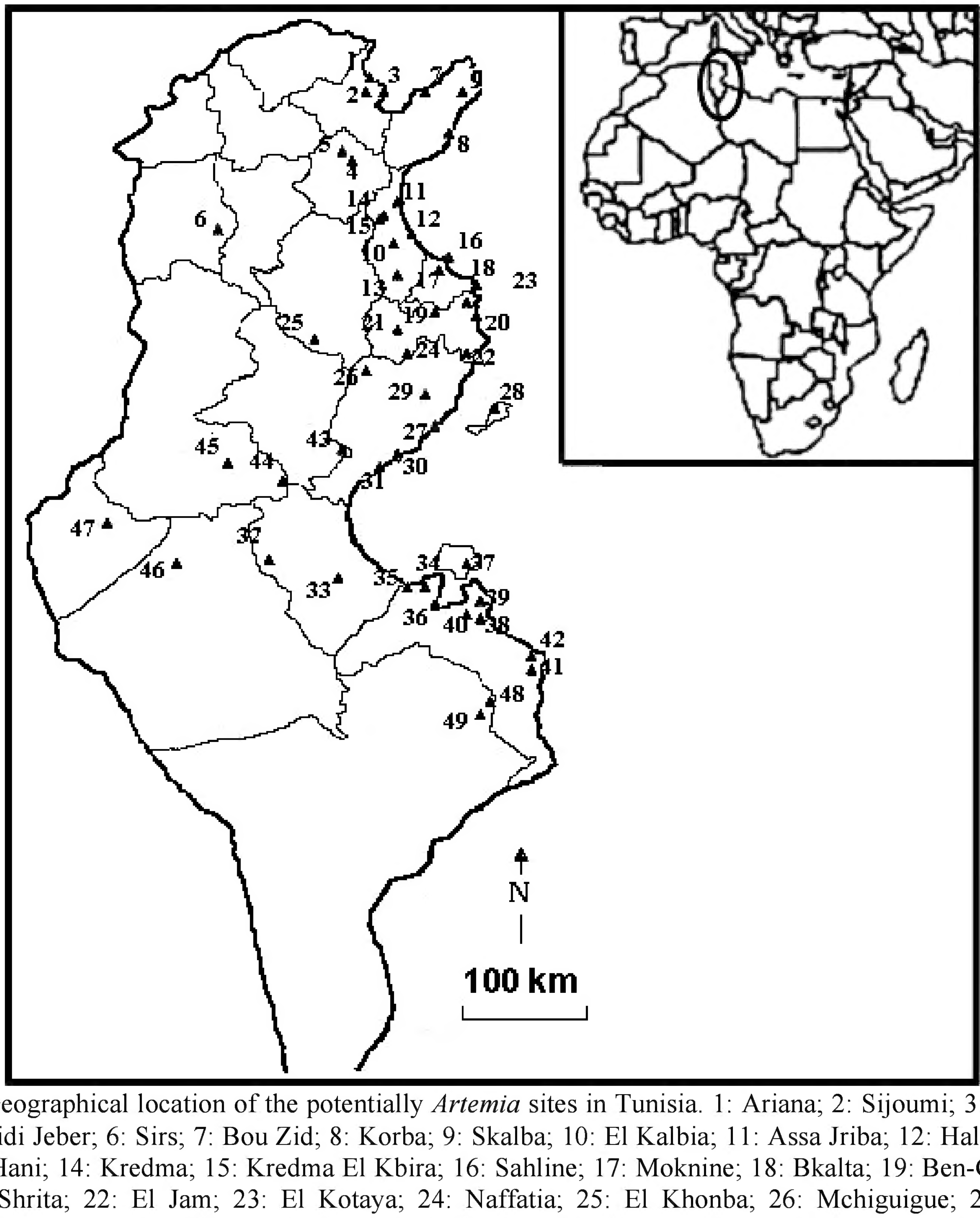

Figure 1. Geographical location of the potentially *Artemia* sites in Tunisia. 1: Ariana; 2: Sijoumi; 3: Megrine; 4: Korzia; 5: Sidi Jeber; 6: Sirs; 7: Bou Zid; 8: Korba; 9: Skalba; 10: El Kalbia; 11: Assa Jriba; 12: Halk El Menzel; 13: Sidi El Hani; 14: Kredma; 15: Kredma El Kbira; 16: Sahline; 17: Moknine; 18: Bkalta; 19: Ben-Ghayada; 20: N'Jila; 21: Shrita; 22: El Jam; 23: El Kotaya; 24: Naffatia; 25: El Khonba; 26: Mchiguigue; 27: Sfax; 28: Abbassia; 29: Boujmal; 30: Wadran; 31: Dreiaa; 32: El-Fjej; 33: Hammet Gabes; 34: El Grine; 35: Jleibya; 36: Mader; 37: El Quastil; 38: El Melah; 39: Zarzis; 40: Mhabeul; 41: El Adhibet; 42: Mnikhra; 43: Noueiel; 44: Sidi Mansour; 45: El Guettar; 46: El Djerid; 47: El Gharsa; 48: Erg El Makhzan; 49: Smara.

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In this work 49 Tunisian inland and costal saline lakes and saltworks were investigated, aiming to inventory the *Artemia* sites (Table 1 and Figure 1). The geographical coordinates were obtained by a GPS receiver (GARMIN GPS 12). Cysts were harvested from the shore of the sites, and adult specimens were collected with a plankton net (150 Ām mesh size). The mating behaviour, the presence or absence of males was recorded *in situ* and after laboratory culturing. For this purpose, nauplii obtained by cyst hatching were made to grow up in 2 l plastic container, with 90 g l^{-1} filtered and autoclaved sea water plus crude sea salt (Amat et al. 2005). Temperature was maintained at 24°C under 16 h light/8 h dark photoperiod. The animals were fed with the unicellular algae *Chlorella* sp. The medium was completely renewed twice a week with fresh microalgae cultures.

In Tunisia, temporary saline lakes represent 29% of the total wetland area, they are formed by 54 Sabkhas (representing 22% of the total wetland area) and 17 chotts (representing 7% of the total wetland area). During our investigation, 21 different locations characterized as temporal or ephemeral catchments (Table 2), distributed over different hydrogeographical zones, were identified as a refuge of *Artemia* (in this work, there are no *Artemia* cysts or adult specimens harvested during our visit to Megrine saltwork. *Artemia* reported in this site was based on the literature and on the cyst collected on 1998 and stored in our laboratory).

The *in situ* study of all the sites where adult *Artemia* were found showed the presence of male and female individuals. This result was confirmed by the laboratory culture (Table 3).

The presence or the absence of *Artemia* at a particular site can have several explanations. *Artemia* cysts can be naturally dispersed over long distances by becoming attached to the feathers or after surviving passage through the digestive system of wading birds (Green et al. 2005) or being carried by wind. They were also deliberately inoculated into salt pans for salt production improvement or for aquaculture purpose (Van Stappen 1996). In those habitats which desiccate completely, salinity fluctuations are extreme. High salinity or desiccation kills adult *Artemia* populations and hence can be the primary factor driving seasonality. However, the prime abiotic factor determining the presence of *Artemia* in these biotopes is high salinity. Although *Artemia* is restricted to hypersaline biotopes, other factors such as temperature, ionic composition and biotic interactions also play an important role in the patterns of its distribution (Van Stappen 2002). Lenz (1987) observed that zooplankton population dynamics are influenced by abiotic factors (salinity, temperature and nutrients concentrations) and by biological interaction (predation, competition and grazers). Moreover, the same author identified two critical factors that determine the population dynamics of *Artemia*: 1) habitat conditions allowing the survival of the animals throughout the year, and 2) predictability of the seasonality of the environment.

It is well known that the brine shrimp *Artemia* can be found in water with different physicochemical characteristics, because of their physiological adaptation to the hostile biotopes. In fact, *Artemia* occurs in the evaporation ponds at salinity levels from 80 to 220g l^{-1} and in rare occasions at salinity up to 340 g l^{-1} (depending on the strain and/or species) (Camargo et al. 2004). Van Stappen (2002) reported that no optimum can be clearly defined for salinity of the *Artemia* environment, for physiological reasons this optimum must be situated towards the lower end of the salinity range, as higher ambient salinity requires higher energy costs for osmoregulation. Moreover, Abatzopoulos et al. (2006) signaled the existence of the parthenogenetic populations in temporary brackish-hypersaline water bodies (lagoon around Urmia Lake, Iran), with salinities as low as 10 g l^{-1}. During this investigation *Artemia* was found at different salinities ranging from 60 to 330 g l^{-1} and different conductivities from 82.3 to 242 ms cm^{-1} (Table 3). Apart from salinity, temperature also affects the distribution pattern of *Artemia* (Vanhaecke et al. 1987). The maximum temperature that *Artemia* populations tolerate has repeatedly been reported to be close to 35 °C. However, this tolerance threshold is strain-dependent (Van Stappen 2002). In fact, Vanheacke et al. (1984) reported that substantial strain differences exist with regard to the resistance for high temperatures: *Artemia salina*

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and *Artemia parthenogenetica* strains did not survive temperatures exceeding 30°C, while 5-10% survival was observed for *Artemia franciscana* from Great Salt Lake (Utah, USA) and San Francisco Bay (San Francisco, USA) cultured at 34°C. In our survey *Artemia* was found at different temperatures ranging from 14 to 26.1°C and lethal temperatures of 30-34°C were never reported at the water surface of the visited lakes (Table 3). Vos (1979) reported that the nauplius growth decreases and the overall appearance of adults deteriorate with pH values below 7.0, and he concluded that the optimum pH for *Artemia* growth ranges from 8.0 to 8.5. In this study different pH values were reported ranging from 7.25 to 8.47 (Table 3).

According to Amat et al. (2005, 2007) *A. franciscana* was proven to be a very successful colonizer, which out-competes the endemic Old World bisexual or parthenogenetic species. More efforts, such as morphological characterization of adult specimens, cultured under standard conditions in order to avoid environmental influences, or biological marker techniques (mitochondrial DNA, polymorphic microsatellite markers) must be invested to characterize autochthonous populations, in order to assess if bisexual *Artemia* present in Tunisian sites belongs to *Artemia salina* or to the invasive American brine shrimp *Artemia franciscana* evidence by Amat et al. (2005, 2007) in the Western Mediterranean region.

Table 1. Tunisian saline sites or biotopes investigated (S. = Sabkha; Sw. = Saltwork; C. = Chott).

Governorate		Site	Geographical coordinates	Date of the last visit	Water depth during our visit
Tunis	1	C. Ariana	36°55’38”N, 10°15’22”E,	April 2006	More than 50 cm
	2	S. Sijoumi	36°46’49”N, 10°07’16”E,	March 2003	More than 50 cm
	3	Sw. Megrine	36°47’N, 10°14’E,	April 2006	More than 50 cm
Zaghouan	4	S. Korzia	36°24’47”N, 09°47’10”E,	March 2006	More than 50 cm
	5	S. Sidi Jeber	36°27’18”N, 09°47’38”E,	March 2006	More than 50 cm
Le Kef	6	Sw. Sirs	-	December 2005	Between 0-50 cm
Nabeul	7	S. Bou Zid	36°52’52”N, 11°07’16”E,	April 2006	More than 50 cm
	8	S. Korba	36°28’47”N, 10°49’04”E,	March 2006	More than 50 cm
	9	S. Skalba	36°49’05”N, 10°56’56”E,	May 2008	Dry
Sousse	10	S. El Kalbia	35°54’41”N, 10°17’48”E,	March 2006	More than 50 cm
	11	S. Assa Jriba	36°14’09”N, 10°26’20”E,	December 2008	Between 10-50 cm
	12	S. Halk El Menzel	36°00’40”N, 10°27’30”E,	March 2007	More than 50 cm
	13	S. Sidi El Hani	35°37’43”N, 10°22’46”E,	March 2006	More than 50 cm
	14	S. Kredma	36°00’38”N, 10°19’43”E,	May 2008	Less than 10cm
	15	S. Kredma El-Kbira	36°00’01”N, 10°20’33”E,	May 2008	Less than 10cm
Monastir	16	Sw. Sahline	35°45’58”N, 10°46’58”E,	February 2006	More than 50 cm
	17	S. Moknine	35°36’20”N, 10°55’37”E,	December 2006	Between 10-50 cm
	18	Sw. Bkalta	35°34’19”N, 11°01’39”E,	April 2007	More than 50 cm
Mahdia	19	S. Ben Ghayada	35°29’00”N, 11°02’55”E,	April 2007	More than 50 cm
	20	S. N’Jila	35°19’43”N, 11°01’48”E,	April 2007	More than 50 cm
	21	S. Shrita	35°20’50”N, 10°16’13”E,	April 2007	Less than 10cm
	22	S. EL Kotaya	35°29’42”N, 11°00’23”E,	May 2008	Between 10-50 cm
	23	S. Naffatia	35°07’06”N, 10°27’12”E,	December 2008	Between 10-50 cm
Kairouan	24	S. El Khonba	35°14’55”N, 10°06’21”E,	April 2007	Between 10-50 cm
Sfax	25	S. El-Jam	35°09’29”N, 10°43’48”E,	December 2008	Between 10-50 cm
	26	S. Mchiguigue	34°57’16”N, 10°02’28”E,	April 2006	Between 10-50 cm
	27	Sw. Sfax	35°45’N, 10°43’E,	March 2005	More than 50 cm
	28	Sw. Abbassia	34°43’27”N, 11°15’00”E,	April 2007	More than 50 cm
	29	S. Boujmal	34°57’53”N, 10°24’04”E,	May 2008	Between 10-50 cm
	30	S. Wadran	34°26’03”N, 10°15’48”E,	April 2007	Between 10-50 cm
Gabes	31	S. Dreiaa	34°10’57”N, 10°00’59”E,	March 2006	Between 10-50 cm
	32	C. El-Fjej	33°52’29”N, 09°04’07”E,	April 2006	Dry

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Governorate		Site	Geographical coordinates	Date of the last visit	Water depth during our visit
	33	S. Hammet Gabes	-	April 2006	Dry
Mednine	34	S. El Grine	33°38’00”N, 10°35’39”E,	December 2008	Dry
	35	S. Jleibya	33°34’41”N, 10°30’02”E,	December 2006	Between 10-50 cm
	36	S. Mader	33°28’08”N, 10°46’48”E,	December 2006	Between 10-50 cm
	37	S. El Quastil	-	April 2006	More than 50 cm
	38	S. El Melah	32°21’34”N, 10°55’22”E,	December 2006	More than 50 cm
	39	Sw. Zarzis	33°24’48”N, 11°03’43”E,	December 2006	More than 50 cm
	40	Sw. Mhabeul	33°24’35”N, 10°51’20”E,	December 2006	More than 50 cm
	41	S. El Adhibet	33°05’42”N, 11°24’29”E,	January 2007	More than 50 cm
	42	S. Mnikhra	33°08’59”N, 11°20’09”E,	April 2007	Less than 10 cm
S. Bouzid	43	S. Noueiel	34°27’28”N, 09°54’51”E,	April 2006	Dry
Gafsa	44	S. Sidi Mansour	34°15’03”N, 09°30’18”E,	March 2006	Between 10-50 cm
Kebeli	45	C. El-Guettar	34°19’44”N, 08°55’12”E,	December 2006	Dry
Tozeur	46	C. El Djerid	33°56’21”N, 08°26’50”E,	December 2006	More than 50 cm
	47	C. El Gharsa	34°09’07”N, 08°04’07”E,	December 2006	More than 50 cm
Tataouin	48	S. Erg El Makhzan	32°02’27”N, 10°51’59”E,	December 2006	More than 50 cm
	49	S. Smara	32°47’04”N, 10°57’36”E,	December 2006	More than 50 cm

Table 2. Geographical characteristics of 21 sites from Tunisia where *Artemia* cysts and/or adult *Artemia* specimens were collected during our investigation. (S. = Sabkha; Sw. = Saltwork; C. = Chott). Last column: (1) Seurat 1921; (2) Gauthier 1928; (3) Dumont et al. 1979; (4) Ben Abdelkader 1985; (5) Sorgeloos et al. 1986; (6) Romdhane 1994; (7) Romdhane et al. 2001; (8) present paper.

Hydro-geographical zone	Site name	Approximate surface (km^2)	Maximum length (km)	Maximum width (km)	Altitude above sea level (m)	First report in literature
Semi arid	C. Ariana	39	8	4	0–50	1
	S. Sijoumi	28-30	9	4	0–50	7
	Sw. Megrine	10	-	-	-	4
	S. Korzia	12	5	3	200	5
	S. Assa Jriba	60	32	2	0-50	8
	Sw. Sahline	12	12	6	0–50	4
	S. Moknine	40	8	4	-	4
	S. Sidi El Hani	350	30	18	-	2
Arid	Sw. Bkalta	1.2	8	0.4	0–1	4
	S. El Jam	30	12	2	50	8
	S. Boujmal	50	15	6	0-50	8
	Sw. Sfax	15	12	-	-	6
	S. Mchiguigue	24	10	2	75	4
	S. Noueiel	110	22	8	50	8
	Sw. Zarzis	1.5	-	-	-	8
	S. El Melah	150	20	12	12	8
	Sw. Mhabeul	3	2	2	0–25	6
	S. Mnikhra	17	8	3	25	8
	S. El Adhibet	125	8	7	25	8
Saharan	C. El Djerid	7000	110	70	10	7
	C. El Ghars	320	50	16	–1	3

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Table 3. Physicochemical characteristics, populations and samples composition of all sites where *Artemia* has been reported in Tunisia. (S. = Sabkha; Sw. = Saltwork; C. = Chott). (+) present; (-) absent; (T) temperature; (S) salinity; *Populations composition was observed in the laboratory culture.

Site	Populations composition		Samples composition		T (°C)	S (g l^{-1})	Conductivity (ms cm^{-1})	pH
	Males	Females	Cysts	Adults				
C. Ariana	+	+	-	+	14	60	82.3	8.47
S. Sijoumi*	+	+	+	-	-	-	-	-
Sw. Megrine*	+	+	-	-	-	-	-	-
S. Korzia	+	+	+	+	19.2	220	193.2	8.16
S. Assa Jriba	+	+	-	+	10	280	211	7.7
Sw. Sahline	+	+	+	+	23.9	120	129.4	8.21
S. Moknine	+	+	+	+	26.1	268	236	7.8
S. Sidi El Hani	+	+	+	+	18.6	270	238	7.73
Sw. Bkalta	+	+	+	+	22.6	290	224	7.92
S. El Jam	+	+	-	+	10.8	55.5	81.8	8.9
S. Boujmal*	+	+	+	-	-	-	-	-
Sw. Sfax*	+	+	+	-	-	-	-	-
S. Mchiguigue	+	+	+	+	25.2	260	230	7.83
S. Noueiel	+	+	-	+	23.1	60	83	8.32
Sw. Zarzis	+	+	+	+	14.4	260	233	8.01
S. El Melah	+	+	+	+	16.6	233	203	7.73
Sw. Mhabeul	+	+	+	+	16	240	218	7.76
S. Mnikhra	+	+	+	+	15.9	86	106.7	7.8
S. El Adhibet	+	+	+	+	16.5	130	156	8.5
C. El Djerid	+	+	-	+	24.8	330	242	7.25
C. El Gharsa	+	+	-	+	22.4	40	56.7	8.6

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